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Full Length Research Paper

In vitro anti-hyperglycaemic effect of glucocapparin isolated from the seeds of *Boscia senegalensis* (Pers.) Lam. ex Poiret

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Glucocapparin (I) used in this study was isolated from the seeds of *Boscia senegalensis* (family Capparidaceae). The structure of (I) was determined on the basis of an extensive analysis of the spectroscopic data. Brine shrimp lethality bioassay of (I) showed a marked significant cytotoxic activity with LC_{50} = 16.482 µg/ml. Compound (I) reduced the liberation of glucose from the liver of rabbits. The active concentration was 30 mg/ml showing that the *in vitro* anti-hyperglycaemic effect shown could be related to the traditional use of *B. senegalensis* seeds in Chad against type 2 diabetes.

Key words: *Boscia senegalensis*, glucocapparin, liver glucose inhibition, cytotoxicity.

INTRODUCTION

Boscia senegalensis (family Capparidaceae) is a common tree in open scrub or savanna woodland, but can also form a thick understorey in woodland and dry forest. This is a Sahelo-Saharan species which occurs below the 20th parallel, while its southern limit runs from Senegal to northern Burkina Faso, the Niger-Nigerian border, the southern bank of lake Chad and western Sudan. It is mainly found under rainfalls of 100 to 300 mm. This plant is an evergreen undershrub or more rarely a shrub, usually 1 to 2 m tall, but sometimes up to 4 m (Arbonnier, 2002). The fruit is often gathered for human consumption; usually very acidic, it becomes edible after soaking in water for about a week. Fruits are frequently sold in the markets for food. It is a regular item of diet in Saharo-Sahelian zone and in Sahel, further south, they are mainly supplementary, but their main value is as an emergency food. Seeds are staple food of the Peuhls from Senegal, but also an important famine food. The fruit is fermented into beer in the Sudan. The leaves are used to protect stored food against parasites (Hans, 2000). According to the African folk medicine, an infusion of leaves is used to remove intestinal parasites from camels. Roots are vermifuge and leaves mixed with millet flour taken each morning on an empty stomach is anthelminthic; dried leaves or dried bark are taken for schistosomiasis. Infusion of the leaves is used as an eyewash and for pruritus of the eye due to syphilis (Orwa et al., 2009; Von Maydell, 1986). In Chad, seeds are used for the treatment of diabetes.

In our previous studies, the hydro alcoholic extracts of the seeds of *B. senegalensis* have been screened for hyperglycaemia induced by oral administration of D(+)-glucose to the albinos rabbits. With the dose of 250 mg/kg, body weight showed a real anti-hyperglycaemic effect. The seeds were characterized by the presence of alkaloids, saponins, tanins and mucilages (Adam et al., 2011).

In leafy twigs, L-stachydrine, hydroxy-3 stachydrine, choline, the sterols β-sitosterol, campesterol,

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stigmasterol, as well as aliphatic alcohols (predominantly C30, C34, C38), carbohydrates and four glycosinolates were detected. Three glucosinolates were identified as methyl, 2-propylisothiocyanate and 2-butylisothiocyanate (Orwa et al., 2009; Bruneton, 1999).

In this paper, we reported on the isolation and characterization of glucocapparin (I) by spectroscopic analyses and we evaluated its toxicity and the level of hepatic liberation of glucose from the liver of rabbits.

MATERIALS AND METHODS

The seeds of *B. senegalensis* were harvested in November 2008, around the Northern part of Ndjamena. The species were identified and voucher specimen was deposited at the "Laboratoire Vétérinaire et Zootechnique de Farcha" (n° 1344). The air-dried materials were then further dried in an oven at 50°C.

Extraction and isolation

The air dried and powdered seed (210 g) of *B. senegalensis* were extracted successively, using a Soxhlet extractor with petroleum ether (750 ml), ethylene trichloride (500 ml) and methanol (750 ml) with intervals of 12 h between each extraction. The different extracts were concentrated using rotary evaporator at a maximum temperature of 45°C. The weight of each dried crude extract was 8.91, 1.35 and 18.79 g, respectively. The methanolic residue was dissolved into a few quantities of the same solvent and refrigerated for one day in the freezer. The amorphous crystals obtained were separated from the mother liquid and washed with pure cold methanol. The white substance was re-crystallized from methanol. It showed a single spot on TLC precoated silica gel 60 F_{254} with the solvent system 15:85 ethyl acetate/hexane. The obtained 2.84 g of crystals were designated as compound I.

Instrumentation

One and two-dimensional NMR spectra were recorded on a BRUKER AV300 spectrometer. Optimal frequencies were 300 MHz for ^{1}H and 75 MHz for ^{13}C. Mass spectra were recorded with a Thermo LCQ Advantage spectrometer. Melting point was determined in capillaries on *Electrothermal*® melting point apparatus.

Brine shrimp cytotoxicity assay

The brine shrimp lethality biossay was performed as described in Meyer et al. (1982) and McLaughlin (1991). Brine shrimp (*Artemia salina* Leach) eggs were hatched in a hatching chamber filled with fresh sea water at 28°C, under conditions of continuous illumination and strong aeration. Separately, 13 mg of compound (I) were dissolved in 10 ml of methanol and from these, 130, 65, 32.5, 16.2, 8.1, 4 and 2 µg/ml were prepared by serial dilution. After 48 h, the larvae (nauplii) were collected. A suspension of 16 nauplii in sea water was added to each sample vial, with a volume of 5 ml and the sample vials were incubated for 24 h at room temperature. After this period, the numbers of nauplii survivors were counted in each sample vial. Larvae were considered dead if they did not exhibit any external movement during several seconds of observation. Pure methanol was used as control. The lethal concentration of compound (I) resulting in 50% mortality of brine shrimp (LC_{50}) were determine from the 24 h counts and dose-response data were transformed into a straight line by means of a trendline fit linear regression analysis (MS Excel version 7). The LC_{50} was derived from the best-fit line obtained. The experiment was carried out three times and the mean of the reading was required.

In vitro study on the hepatic liberation of glucose

Based on the procedure of the washed liver achieved by Claude Bernard (Grmek, 1997) and used elsewhere (Dods, 2003; Tietz, 1995; Trinder, 1969), a rabbit was sacrificed; the abdominal cavity was incised, the liver was taken out, immediately weighed and dropped in a flask containing the standard physiological solution at pH 7.4 (Mac Ewen solution). Then, the liver was cut into pieces of 300 mg average weight and washed with the Mac Ewen solution. Each piece was dropped in five different solutions: Solution A: 1 ml of the Mac Ewen (control); Solution B, C, D, E, F: 1 ml of the Mac Ewen + a drop of emulsifier + 10, 20, 30, 40, 50 mg/ml of compound (I), respectively.

Each concentration was tested 5 times. The different solutions were homogenized and incubated for 20 min at room temperature. After this period, 10 µl of each sample were taken out with a micropipette and placed in a cell. In this volume, was added 1 ml of the enzymatic reagent whose composition was as follows: phosphate buffer pH 7.4: 13.8 mmol/L, phenol: 10 mmol/L, 4-aminoantipyrine: 0.3 mmol/L, glucose oxydase: ≥10 000 U/L and peroxydase: ≥700 U/L (GLUCOSE PAP SL, SAS, SPPIM, VOIT, FRANCE). The whole sample-reagent was then homogenised and incubated for 15 min. The concentrations of glucose at different times, from 0 to 120 min were determined after the enzymatic oxidation by the measurement of the optical density at λ = 500 nm using a spectrophotometer DREL 2400 HACH.

Data analysis

All the data reported were expressed as mean ±S.E.M. Statistical analysis were performed using Statistica Statsoft V 5.5. The values were considered to be significantly different when the p value was less than 0.05 as compared to the respective control.

RESULTS AND DISCUSSION

Chemical characterization

Compound (I), crystals m.p. 204 to 205°C, yielded 1.35% of the dry material. It showed on thin layer chromatography (TLC) a spot with Rf = 0.52. The negative mode ESI mass spectrum showed a peak at 332 $[M-H]^-$, corresponding to the molecular formula $C_8H_{15}O_9S_2$. It showed also peaks at m/z 664.7, 674 and 687, corresponding to the fragments $[2M-H]^-$, $[2M+Na - CH_3]^+$ and $[2M+Na - 2H]^+$, respectively. ^{1}H and ^{13}C spectra exhibited signals from 3 to 5 ppm and at 60 to 105 ppm, respectively which are characteristics of protons and carbons of a sugar unit. The doublet of the anomeric proton, H-1', at 4.74 ppm, had a coupling constant of 7.2 Hz, revealing a β-linked glycoside moiety (Iori et al., 2008; Kiddle et al., 2001; Reichelt et al., 2002). The work of carbons and protons, achieved by HMBC and HMQC 2D shift correlations (Table 1), led to the confirmation of the structure of β-D-glucopyranosyl unit (Kundu et al.,

Table 1. ^{1}H-NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectra data of compound (I).

Position	δ_C	δ_H (J, Hz)	HMBC
Aliphatic moiety			
1	154.2		
2	18.2	2.24 s	C1, C1'
-OSO_3H		4.77	
Glucose moiety			
1'	81.9	4.74 d(9.8)	C2', C3', C1
2'	72.7	3.05	C3'
3'	78.0	3.22	C4'
4 '	69.9	3.06	C3', C5'
5'	81.1	3.21	C1', C4', C6'
6'a	61.0	3.68 brd (12.1)	C5'
6'b		3.36 m	C5'
C'$_2$-OH		5.50 d (4.8)	
C'$_3$-OH		5.18 brs	
C'$_4$-OH		5.06 brs	
C'$_6$-OH		4.72 brs	

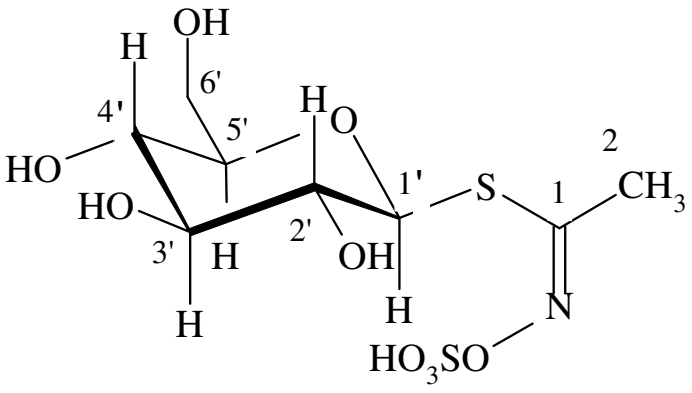

Figure 1. Structure of glucocapparin (I).

1993). Extra signals on the ^{1}H and ^{13}C spectra were assigned to an aliphatic moiety. Detailed analysis of this second set of signals allowed characterization of a broad singlet at δH 4.77 ppm related to the presence of sulfonyl acid moiety and a methyl singlet (δH 2.24 ppm, δC 18.2 ppm). On the basis of the above spectral data and by comparison with the literature (Montaut et al., 2009), compound (I) was identified as methylglucosinolate or glucocapparin (Figure 1). To the best of our knowledge, this is the first report on its isolation and characterization from the seeds of *B. senegalensis*, although this compound was previously obtained from other plants species (Dauvergne et al., 2006; Reichelt et al., 2002). The amount of this glucosinolate in the seeds of *B. senegalensis* is very large when compared with the quantity of other glucosinolates, such as glucocochlearin isolated from *Cochlearia genus* (Dauvergne et al., 2006).

Biological effects

Significant lethality to *A. salina* L was observed with exposure to different dose levels of compound (I). The degree of lethality was directly proportional to the concentration of glucocapparin. Maximum mortality took place at 130 µg/ml whereas least mortalities were at 4 µg/ml. In other words, mortality increased gradually with the increase in concentration of glucocapparin (Table 2). The lethal concentration LC_{50} was obtained from the best-fit line slope and found to be LC_{50} = 16.482 µg/ml (Figure 2). In toxicity evaluation of plant extracts by brine shrimp lethality bioassay, LC_{50} values lower than 1000

Table 2. Brine shrimp bioassay of glucocapparin (I).

Concentration (µg/ml)	130	65.5	32	16	8	4	2
Number of shrimp per test sample	16	16	16	16	16	16	16
Number of survivors	0	1	3	7	11	15	16
Number of deaths	16	15	13	9	5	1	0
Percentage mortality	100	93.75	68.75	43.75	18.75	6.25	0

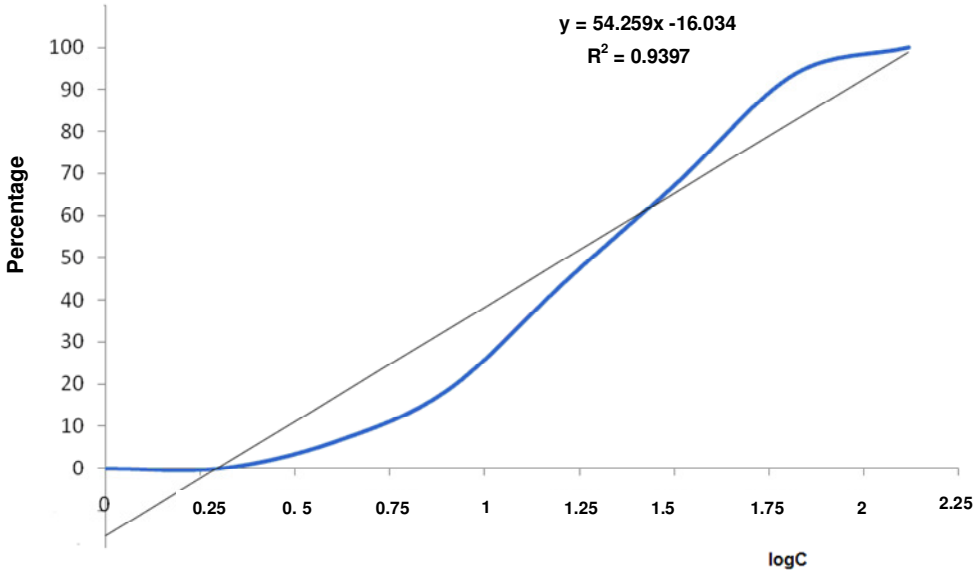

Figure 2. Plot of log of toxicant concentration (mg/ml) versus shrimp mortality (%).

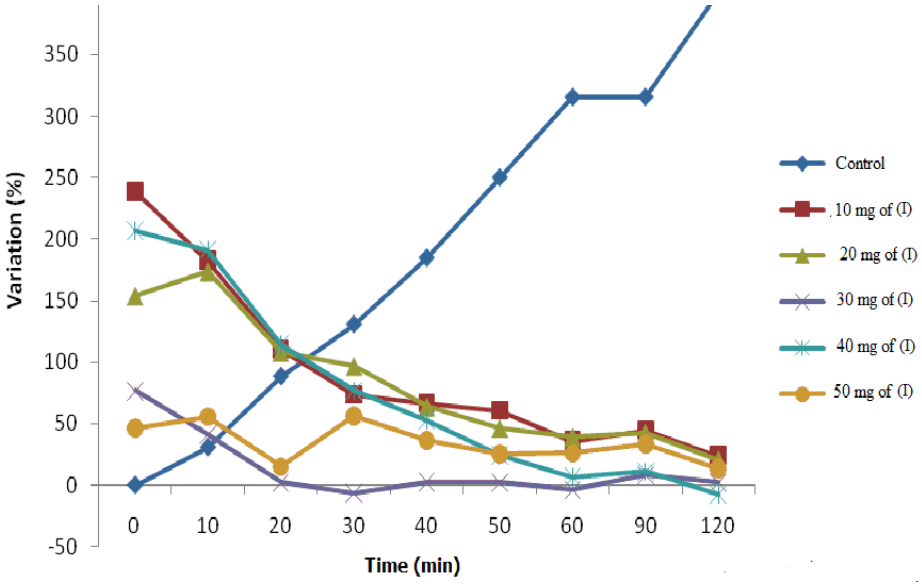

Figure 3. Effect of the concentration of glucocapparin (I) on the variation of optical density (the hepatic liberation of glucose).

µg/ml are considered bioactive (Meyer et al., 1982). The significant lethality to brine shrimp showed that glucocapparin is a potent cytotoxic component.

Results from *in vitro* study of the effect of compound (I) on the hepatic liberation of glucose are given in Figure 3. In the control solution, the liver isolated from the rabbit,

continued to liberate glucose; the level of glucose increased from 30.76% at the 10th min to 400% at the 120th min. When compound (I) was added, different doses induced the reduction of glucose level. It can be seen that the dose of 30 mg/ml reduced better the production of glucose from the liver. This is the first report on the *in vitro* antihyperglycaemic effect of the purified glucocapparin.

In conclusion, this study shows that the seeds of *B. senegalensis* are a valuable source of glucocapparin. This component which presents an interesting anti-hyperglycaemic effect *in vitro* could be related to the traditional use of the seeds in Chad, against type 2 diabetes. However, the cytotoxicity effect pointed out suggests that further investigations extended to *in vivo* would be needed to make the glucocapparin a potential anti-diabetic drug.

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